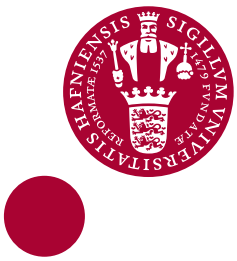

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High-resolution Assays Combined with HPLC-HRMS-SPE-*tt*NMR for Identification of Antidiabetic Compounds in root of *Scutellaria Baicalensis*

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Abstract

This work describes an analytical platform based on high-resolution radical scavenging and high-resolution α-glucosidase inhibition assays in combination with hyphenation of high-performance liquid chromatography, high-resolution mass spectrometry, solid-phase extraction, and tube-transfer nuclear magnetic resonance spectroscopy, *i.e.*, HPLC-HRMS-SPE-*tt*NMR/high-resolution radical scavenging and high-resolution α-glucosidase assays. The platform enables fast screening of complex matrices for individual analytes with α-glucosidase inhibitory and radical scavenging activity, followed by structural indentification targeted the active analytes only.

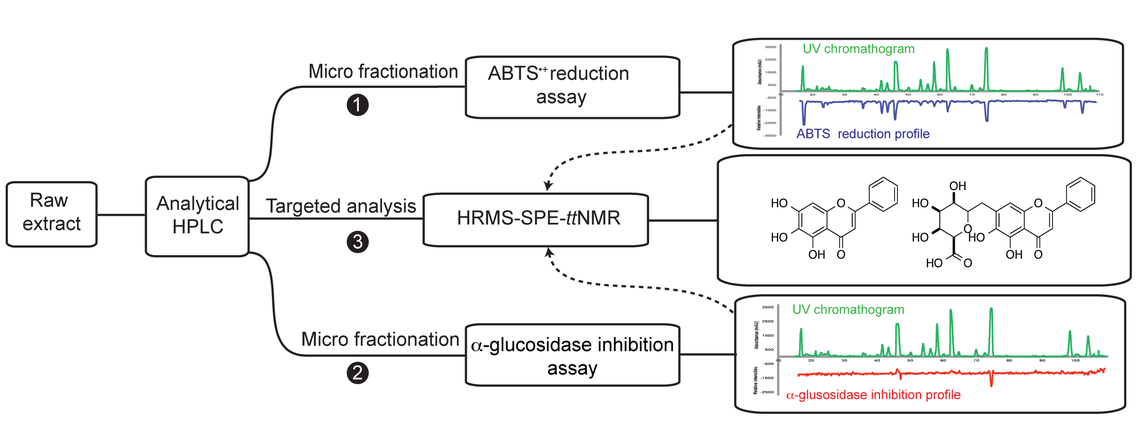
Introduction

Type 2 diabetes is one of the most prevalent diseases affecting 246 million worldwide and its incidence and serious complications continue to grow rapidly. Patients with type 2 diabetes suffer from a series of micro- and macrovascular complications such as visual impairment, blindness, neuropath, kidney failure and cardiovascular diseases.

Radix Scutellaria is the dried root of the medicinal plant *Scutellaria baicalensis*. *Radix Scutellaria* is officially listed in the Chinese Pharmacopeia and Japanese Pharmacopoeia, and exhibits a variety of therapeutic effects and has long history of application in traditional formulation and in modern herbal medication – and is also used as a food additive.

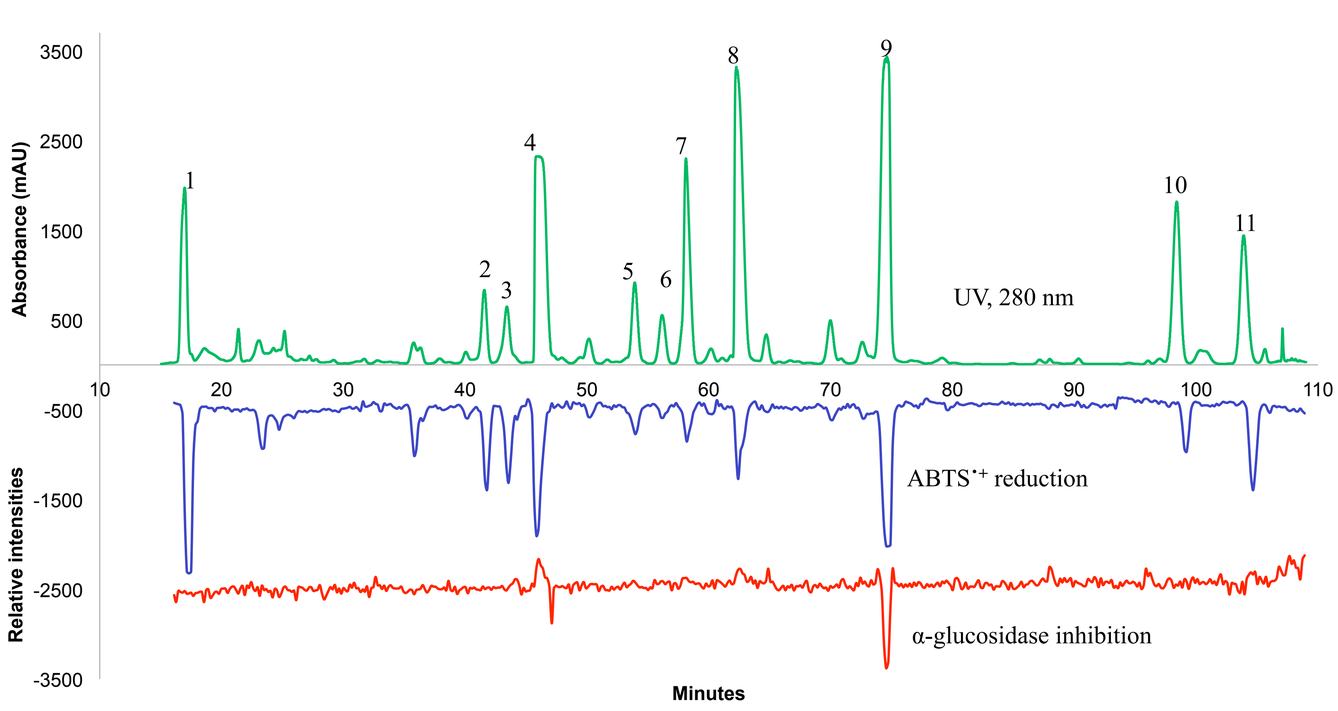
Method

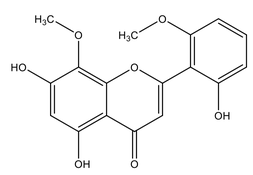
Figure 1. Schematic represenation of HPLC-HRMS-SPE-*tt*NMR analysis of *Radix Scutellaria* extract guided by high-resolution $ABTS^{\bullet+}$ reduction and α-glucosidase inhibition profiles. **Path 1:** microfractionation into six 96-well microplates followed by $ABTS^{\bullet+}$ reduction assay of each microfraction to produce high-resolution $ABTS^{\bullet+}$ reduction profile. **Path 2:** microfractionation into six 96-well microplates followed by α-glucosidase inhibition assay to produce high-resolution α-glucosidase inhibition profile. **Path 3:** HPLC-HRMS-SPE-*tt*NMR analysis targeting antioxidants and α-glucosidase inhibitory metabolites identified in the preceeding procedures.



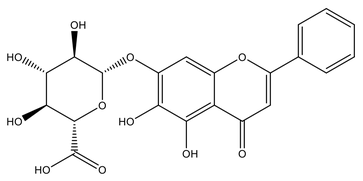
Results

Figure 2. High-resolution α-glucosidase inhibition and radical scavenging profiles of *Radix Scutellaria* with overlaid HPLC chromatogram at 280 nm. Peaks are numbered sequentially with increasing elution order. The radical scavenging and α-glucosidase inhibition profiles provide good resolution that allows disclosure of the individual metabolites responsible for the observed activities, that is, ***correlating peaks in the biochromatogram with individual peaks in the overlaid HPLC chromatograms***.

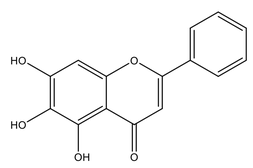




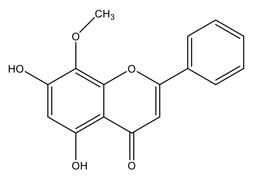
Peak 2. Viscidulin III



Peak 4. Baicalin



Peak 9. Baicalein



Peak 11. wogonin

Concluding Remarks

This work describe the development of a bioassay-coupled HPLC-HRMS-SPE-*tt*NMR platform for identification of radical scavengers and α-glucosidase inhibitors in an extract of *Radix Scutellaria*. The biochromatogram can be used to pinpoint HPLC-analytes with bioactivity.

Acknowledgments

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